

## Tracing the effects of Sefidrud dams on *Capoeta gracilis* (Cyprinidae) populations using Truss distances in southern Caspian Sea basin

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**Abstract:** It is postulated that building of Manjil and Tarik dams on Sefidrud River has led to morphological divergence of Siah Mahi, *Capoeta gracilis* of up-and downstream populations due to isolation. To test this, a total of 97 individuals of *C. gracilis* were collected from three sampling sites, including upstream of Manjil dam, upstream of Tarik dam and downstream of Tarik dam, in November 2013. A 13-landmark morphometric truss network system was used to investigate the hypothesis. Principal component analysis (PCA), canonical variates analysis (CVA), linear discriminant function analysis (DFA) and clustering analysis (CA) were used to examine morphological differences among the populations. Univariate analysis of variance showed significant differences among the means of the three groups for 48 standardized morphometric measurements out of 78 characters studied. In, DFA the overall assignment of individuals into their original groups was 77.8%. The PCA scatter plot of individual component scores between the first and second factors showed 97 fish specimens grouped into three areas but with a relatively low degree of overlap among the three populations. Cluster analysis indicating different populations of *C. gracilis* in up- and downstream Manjil and Tarik dams in Sefidrud River. The present study indicated the presence of morphologically different populations of *C. gracilis* in up and downstream of Manjil and Tarik dams in Sefidrud River, probably, is due to their limited downstream dispersal and elimination of upstream migration altogether, due to the construction of the dams.

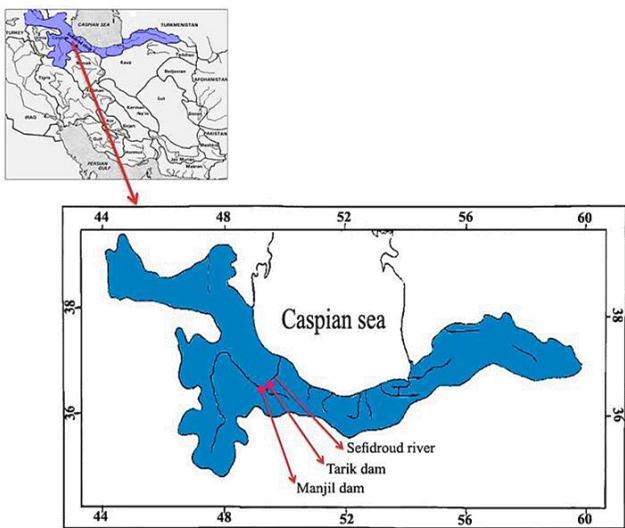
**Keywords:** Siah Mahi, Manjil Dam, Tarik Dam, Morphological Divergence.

### Introduction

Construction of large dams which has always been regarded as a development index and is often accompanied by impacts on the environment and local communities (Carvalho & Hauser 1994; Heywood 1995; Yamamoto et al. 2006), especially aquatic animals. Many freshwater fish species are typically threatened by direct and indirect effects of human activities, such as habitat loss and fragmentation (Yamamoto et al. 2006; Anvarifar et al. 2011). Biodiversity is ultimately based on genetic diversity, which is likely to be affected by habitat fragmentation, one of the most pervasive negative man-induced processes for nature conservation

(Heywood 1995; Yamamoto et al. 2006). It is suggested that fragmentation of river ecosystems may result in the conversion of migration patterns among fish populations, producing 'genetic stocks' that are reproductively isolated units and are genetically different from other stocks (Carvalho & Hauser 1994).

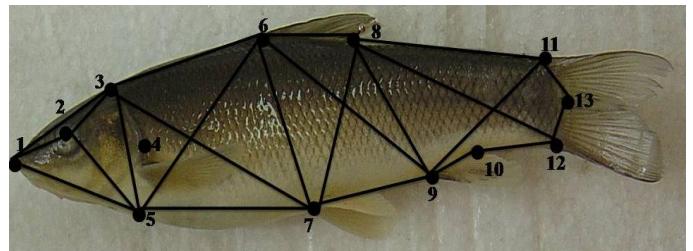
Sefidrud River is one of the most important rivers in the southern Caspian Sea basin (Esmaeili et al. 2010; Coad 2014) with 765 km in length. The Manjil and Tarik dams were constructed on Sefidrud River in 1962 and 1968, respectively (Najmaii 2004), which have effectively fragmented the river into upstream and downstream and have probably



**Fig.1.** Map of Caspian Sea basin showing sampling sites.

blocked the migration of the fish among up and downstream. Manjil Dam (also known as the Sefidrud Dam) is a buttress dam on the Sefidrud River near Manjil (Guilan Province, Iran). It was constructed to store water for irrigation and produce hydroelectric power. It is 106 m high and forms a reservoir with a capacity of 1.82 km<sup>3</sup>. Tarik Dam is located 35 km downstream from Manjil Dam and diverts releases from Manjil Dam for irrigation (Najmai 2004).

Various effects of the dams on fish population have been documented in the recent years (Dakin et al. 2007; Yamamoto et al. 2006; Anvarifar et al. 2011). The long-term isolation of populations and interbreeding can lead to morphometric differentiations between populations, and provide a basis for population differentiation (Yamamoto et al. 2006; Anvarifar et al. 2011). Morphometric characters have been successfully used for stock identification. A new system of morphometric measurements is truss network system that has been increasingly used for stock identification (Strauss & Bookstein 1982; Turan 1999; Turan et al. 2005). This system covers the entire the body shape of fish in a uniform network, and theoretically should increase the likelihood of extracting morphometric differences between stocks. A regionally unbiased network of morphometric measurements over the 2



**Fig.2.** Locations of the 13 landmarks for constructing the truss network on *C. gracilis*. 1. Tip of snout; 2. Center of eye; 3. Forehead (end of frontal bone); 4. End of operculum; 5. Dorsal origin of pectoral fin; 6. Origin of dorsal fin; 7. Origin of pelvic fin; 8. End of dorsal fin; 9. Origin of anal fin; 10. End of anal fin; 11. Dorsal side of caudal peduncle, at the nadir; 12. Ventral side of caudal peduncle, at the nadir; 13. End of lateral line.

dimensional outline of a fish should give more information about local body differences than a conventional set of measurements (Turan 1999; Akbarzadeh et al. 2009).

One of the predominant fish in Sefidrud River is Siah Mahi, *Capoeta gracilis* (formerly known as *Capoeta capoeta gracilis*). In addition to its ecological significance, *Capoeta gracilis* is an important species being harvested for food and used in sport fishing by local people (Kiabi et al. 1999). There is only one report on the impact of dams on its morphology in this river (i.e., Heidari et al. 2013). The present study was aimed at assessing the impact of construction of Manjil and Tarik dams on morphometric traits of *C. gracilis* and examining if specific ecological constraints (due to habitat variation), have influenced the population of *C. gracilis* to differentiate it into the various stocks.

## Materials and Methods

A total of 97 individuals of *C. gracilis* were collected from three sampling sites, one sample from upstream of Manjil dam (36°77'89"N, 49°15'25"E; 40 individuals, station 1), another one from downstream of Manjil dam that is upstream of Tarik dam (36°46'52.86"N, 49°61'18"E; 24 individuals, station 2) and the third one from downstream of Tarik dam (36°99'15"N, 49°57'71"E; 33 individuals, station 3), in November 2013 by electrofishing with 200–300V (Fig. 1).

**Table 1.** Descriptive data [mean  $\pm$  SD standard length (mm) and body weight (g)] of *C. gracilis* from upstream and downstream of Manjil and Tarik dams in Sefidrud River.

Locality	n	Standard length		Body weight	
		Min-max	Mean $\pm$ SD	Min-Max	Mean $\pm$ SD
Upstream of Manjil Dam	40	31.02-133.27	81.56 $\pm$ 28.64	2.80-45.50	14.26 $\pm$ 12.04
Downstream of Manjil Dam	24	88.16-185.00	146.81 $\pm$ 25.83	15.40-162.60	77.85 $\pm$ 39.29
Downstream of Tarik Dam	33	56.49-141.70	77.53 $\pm$ 18.13	4.60-61.00	11.64 $\pm$ 10.41

The specimens were fixed into 10% formaldehyde at the sampling sites and transported to the Laboratory for further studies. Then a total of 78 distance measurements between 13 landmark points were measured using the truss network system according to Strauss & Bookstein (1982) with minor modifications (Fig. 2).

Measurements of specimens are made by collecting X-Y coordinate data for relevant morphological features, and followed by the three-step process as described below (Turan 1999). The fish were placed on a white board with dorsal and anal fins held erect by pins. The right body profile of each fish was photographed with a 300-dpi, 32-bit color digital camera (Cybershot DSC-F505; Sony, Japan). Images were saved in jpg format and analyzed with TPSdig2 (v.2.04; Rohlf 2005) to coordinates of 13 landmark points. A box truss of 26 lines connecting landmark points were generated for each fish to extract the basic body shape (Cardin & Friedland 1999). All data were transferred to a spreadsheet file (Excel 2010), and the X-Y coordinate data transformed into linear distances by computer for subsequent analysis (Turan 1999).

To remove size dependent data from raw truss data, they were corrected by adapting an allometric method based on Elliott et al. (1995) using  $M_{adj} = M (L_s / L_0)^b$  formula, where  $M$  is the original measurement,  $M_{adj}$  the size adjusted measurement,  $L_0$  the standard length of the fish,  $L_s$  the overall mean of the standard length for all fish from all samples in each analysis, and  $b$  was estimated for each character from the observed data as the slope of the regression of  $\log M$  on  $\log L_0$  using all fish in any group. The derived data from the allometric method were confirmed by testing significance of the correlation

**Table 2.** Age composition of *C. gracilis* from upstream and downstream of Manjil and Tarik dams in Sefidrud River.

Age (years)	Upstream Manjil dam	Downstream Manjil dam	Downstream Tarik dam
1+	16	-	2
2+	13	1	27
3+	9	12	4
4+	2	11	-
Total	40	24	33

between transformed variables and standard length (Turan 1999). To evaluate the statistical significance of individual morphological characters among the groups, Univariate Analysis of Variance (ANOVA) was performed for each morphological character (Zar 1984). For determining adequacy of samples size, the ratio of the number of organisms measured (N) relative to the parameters included (P), was calculated. Authors of theoretical works on PCA and DFA recommended that the N:P ratio in the analysis should be at least 3–3.5 (Johnson 1981). Principal component analysis (PCA) helps in morphometric data reduction (Veasey et al. 2001), to decrease the redundancy among the variables and extract a number of independent variables for population differentiation (Turan 1999; Samaee et al. 2009; Veasey et al. 2001). The Wilks' Lambda was used to compare the difference between studied groups. The discriminant function analysis (DFA) was used to calculate the percentage of correctly classified (PCC) fish. A cross-validation using PCC was carried out to estimate the expected actual error rates of the classification functions. In addition, canonical variate analysis (CVA) was applied to compare studied populations based on morphological data. As a complement to discriminant analysis, morphometric distances among the individuals of the three groups

**Table 3.** The results of ANOVA for morphometric measurements of *C. gracilis* from upstream and downstream of Manjil and Tarik dams in Sefidrud River.

Characters	F	P	Characters	F	P	Characters	F	P
1-2	2.949	0.058	3-8	4.553	0.013	6-10	5.143	0.008
1-3	3.142	0.048	3-9	2.957	0.057	6-11	7.556	0.001
1-4	6.057	0.003	3-10	1.041	0.358	6-12	13.730	0.000
1-5	3.596	0.032	3-11	5.065	0.008	6-13	6.444	0.002
1-6	0.705	0.487	3-12	2.350	0.101	7-8	7.012	0.002
1-7	2.840	0.064	3-13	2.826	0.065	7-9	10.891	0.000
1-8	3.657	0.030	4-5	0.403	0.670	7-10	3.461	0.050
1-9	4.004	0.022	4-6	3.390	0.038	7-11	2.672	0.075
1-10	1.698	1.89	4-7	4.108	0.020	7-12	0.470	0.627
1-11	5.617	0.005	4-8	6.547	0.002	7-13	2.784	0.067
1-12	0.779	0.462	4-9	8.908	0.000	8-9	9.410	0.000
2-3	0.26	0.975	4-10	4.524	0.014	8-10	6.745	0.002
2-4	6.679	0.002	4-11	7.464	0.001	8-11	12.167	0.000
2-5	7.530	0.001	4-12	2.739	0.070	8-12	19.473	0.000
2-6	2.638	0.077	4-13	6.676	0.002	8-13	12.671	0.000
2-7	5.835	0.004	5-6	6.343	0.003	9-10	3.139	0.048
2-8	4.050	0.021	5-7	3.577	0.032	9-11	9.096	0.000
2-9	2.289	0.074	5-8	4.088	0.020	9-12	2.935	0.058
2-10	0.542	0.584	5-9	8.397	0.000	9-13	1.111	0.334
2-11	6.846	0.002	5-10	3.438	0.037	10-11	3.100	0.050
2-12	1.717	0.186	5-11	9.192	0.000	10-12	2.350	0.101
2-13	2.802	0.066	5-12	2.721	0.071	10-13	0.425	0.655
3-4	10.881	0.000	5-13	6.025	0.004	11-12	23.030	0.000
3-5	17.124	0.000	6-7	27.908	0.000	11-13	2.564	0.083
3-6	3.258	0.043	6-8	1.762	0.178	12-13	0.293	0.746
3-7	6.819	0.002	6-9	4.034	0.021			

were calculated for cluster analyzes (CA) (Veasey et al. 2001) by adopting the Euclidean square distance as a measure of dissimilarity and the UPGMA method as the clustering algorithm (Sneath & Sokal 1973). Statistical analyses for morphometric data were performed using the SPSS software (version 16).

## Results

The mean and standard deviation (SD) of length and weight of the sampled specimens were calculated (Table 1). The age of the specimens ranged from 1<sup>+</sup> to 4<sup>+</sup> years (Table 2).

Statistically significant differences among upstream and downstream populations of Siah Mahi in Manjil and Tarik dams in Sefidrud River were observed in 48 morphometric characters out of 78 standardized characters (Table 3). Of these 48 characters, 32 characters were found to be highly significant ( $p<0.01$ ) and were used further for

multivariate analysis. This traits including 1-4, 1-11, 2-4, 2-5, 2-7, 2-11, 3-4, 3-5, 3-7, 3-11, 4-8, 4-9, 4-11, 4-13, 5-6, 5-9, 5-11, 5-13, 6-7, 6-10, 6-11, 6-12, 6-13, 7-8, 7-9, 8-9, 8-10, 8-11, 8-12, 8-13, 9-11 and 11-12. The analysis of variance revealed significant phenotypic variation between the three populations (Table 3).

The morphometric characters did not differ significantly ( $p>0.05$ ) between the sexes, therefore, the data for both sexes were pooled for all subsequent analyses. In this study the N:P ratio was 3.03 (97/32) and used only from morphometric characters that were significant at a high level ( $p<0.01$ ). In other words, to determine which morphometric measurement most effectively differentiates populations, the contributions of variables to principal components (PC) were examined. To examine the suitability of the data for PCA, Bartlett's Test of sphericity and the Kaiser–Meyer–Olkin (KMO) measure was performed. The Bartlett's Test

**Table 4.** Eigenvalues, percentage of variance explained for morphometric measurements for *C. gracilis* populations from upstream and downstream of Manjil and Tarik dams in Sefidrud River.

Eigenvalues	Percentage of variance	Percentage of cumulative variance
10.188	31.837	31.837
7.331	22.910	54.747
3.143	9.822	64.569
2.512	7.851	72.420
1.827	5.710	78.130
1.402	4.381	82.511
1.243	3.884	86.395

**Table 5.** Percentage of specimens classified in each group and after cross validation for morphometric measurements for *C. gracilis* upstream and downstream of Manjil and Tarik dams in Sefidrud River.

Area	Upstream Manjil dam	Downstream Manjil dam (upstream Tarik dam)	Downstream Tarik dam
<b>Original</b>			
Upstream of Manjil dam	87.5	7.5	5
Downstream of Manjil dam	17.6	47.1	35.3
Downstream of Tarik dam	6.1	12.1	81.8
<b>Cross-validated</b>			
Upstream of Manjil dam	85	10.0	5
Downstream of Manjil dam	17.6	47.1	35.3
Downstream of Tarik dam	6.1	12.1	81.8

of sphericity can be used to determine if the value of the correlation matrix is equal to zero and the KMO measure of sampling adequacy tests whether the partial correlation among variables is sufficiently high (Nimalathasan 2009). The KMO statistics varies between 0 and 1. Kaiser (1974) recommends that values greater than 0.5 are acceptable. In this study, the value of KMO for overall matrix is 0.6 and the Bartlett's Test of sphericity is significant ( $p<0.01$ ). The results of KMO and Bartlett's suggest that the sampled data is appropriate to proceed with a factor analysis procedure. Principal component analysis of 32 morphometric measurements extracted 7 factors with eigenvalues  $>1$ , explaining 86.395% of the variance (Table 4).

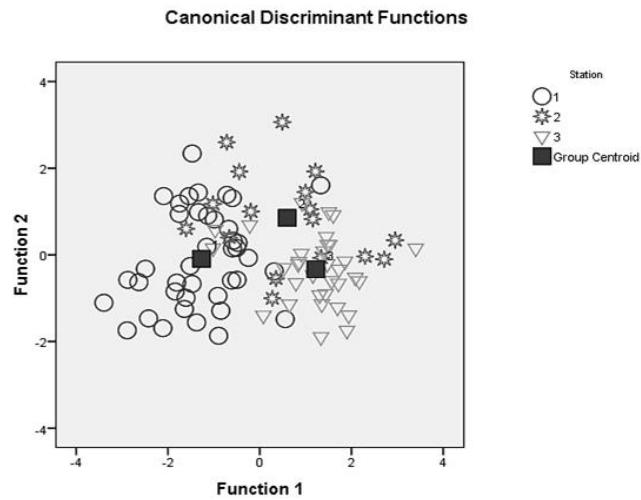
The first principal component (PC1) accounted for 31.837% of the variation and the second principal component (PC2) for 22.910% (Table 4) and the most significant loadings on PC1 were 1-4, 1-11, 2-4, 2-5, 2-7, 2-11, 3-7, 3-11, 4-8, 4-9, 4-11, 4-13, 5-6, 5-9, 5-11, 5-13, 6-7, 6-11, 6-12, 6-13, 8-9, 8-10, 8-11, 8-12, 8-13 and on PC2 were 1-4, 2-4, 2-5, 3-4, 3-

5, 4-8, 4-9, 4-13, 5-9, 5-13, 6-7, 6-11, 6-12, 6-13, 7-9, 8-11, 8-12, 8-13, 9-11, 11-12. In this analysis, the characteristics with an eigenvalues exceeding 1 were included and others discarded. CVA analysis, clearly revealed that the populations of up and downstream dams, were different from each other (Fig. 4).

Result of Wilks' Lambda tests of discriminant analysis for verifying differences among studied populations indicated significant differences in morphometric characters of the three populations. Wilks' Lambda scores for function 1 through 2 and function 2 were 0.354 and 0.841, respectively. In this test, both two functions were highly significant ( $p<0.01$ ).

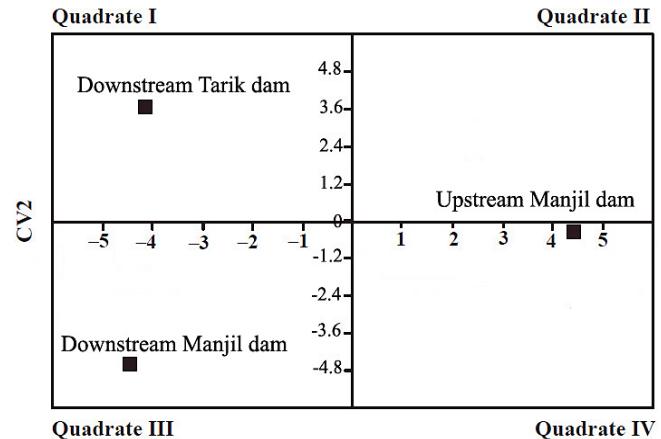
The linear discriminant analysis gave an average PCC (Percentage of specimens classified) of 77.8% for morphometric characters indicating a high rate of correct classification of individuals into their original populations (Table 5).

The dendrogram derived from CA of Euclidean square distances among individuals of centroids showed three detectable populations with some

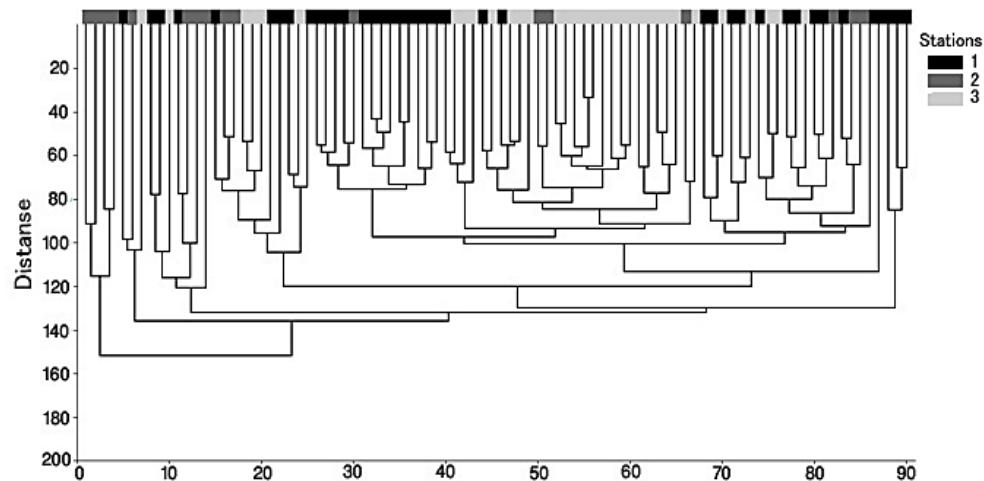


**Fig.3.** DFA Plot of morphometric measurements for *C. gracilis* from upstream and downstream of Manjil and Tarik dams in Sefidrud River (1: Upstream Manjil Dam, 2: Downstream Manjil Dam, 3: Downstream Tarik Dam).

populations are able to persist in these river stretches (Maia et al. 2007). The *C. gracilis* populations in



**Fig.4.** CVA Plot of morphometric measurements for *C. gracilis* from upstream and downstream of Manjil and Tarik dams in Sefidrud River.



**Fig.5.** Dendrogram derived from cluster analyses of morphometric measurements on the basis of Euclidean distance for *C. gracilis* in upstream and downstream of Manjil and Tarik dams in Sefidrud River (1: Upstream Manjil dam, 2: Downstream Manjil dam and 3: Downstream Tarik dam).

overlaps, indicating the presence of three morphologically different populations of *C. gracilis* in up and downstream of Manjil and Tarik dams on Sefidrud River (Fig. 5).

## Discussion

At present, more than 490 hydroelectric dams are in operation but only a few publications have focused on the consequences of the loss of river connectivity for the migratory fish populations. Some of these studies show that when there are favorable conditions in the upstream of a dam, at least a few fish

Sefidrud River are limited to a very short stretch of appropriate habitat, with limited high differentiation between the upstream and downstream populations, and are probably suffering from the effects of inbreeding.

The present study reveals that the Manjil and Tarik dams on Sefidrud River have probably created different populations of *C. gracilis* in upstream and downstream of the dams. It can be caused by limited downstream dispersal of fish, and elimination of upstream migration altogether (Dakin et al. 2007). The dams obstruct the upward migration of fish

especially that of the migratory species resulting in an ecological trap for migratory fish that ascend the fish passages (Pelicice & Agosinho 2008). After damming the river, fish populations were isolated but did not evolve or phenotypically adapt to their new environments and hence did not express different growth characteristics. Although in some cases it is clear that genetic isolation is responsible for intraspecific differences in growth patterns, additive effects of environmental conditions (e.g. temperature, density of food and diseases) are also important (Longhurst & Pauly 1987).

The fragmentation of river can lead to an inter-population structuring assessed by the differentiation of morphometric characteristics. The analysis of variance revealed significant phenotypic variation between the three studied populations and DFA results indicating a high differentiation between the populations of Siah Mahi in the study area. Also graphs of function 1 and function 2 scores for each sample revealed the three populations were distinct from each other. These morphological differences may be solely related to body shape variation and not to size effects; size-related traits play a predominant role in morphometric analysis and the results may be erroneous, if not adjusted for statistical analyses of data (Tzeng 2004). In the present study, the significant differences among the populations are due to the body shape variation and the size effect was removed successfully by allometric transformation. Construction of a dam on a river can block the fish movements, especially the upstream movement (McAllister et al. 2001) and by dramatic changes affecting fish communities.

As the studied populations were caught from different ecosystems in upstream and downstream of these dams, the detected differentiations among them may result from different environmental and habitat conditions, such as temperature, turbidity, food availability, and water depth and flow. Of course, in order to confirm these causal hypotheses, more detailed data on the environmental conditions from each sampled sites would be required.

In conclusion the present study indicates that there are morphologically different populations of *C. gracilis* in upstream and downstream of Manjil and Tarik dams on Sefidrud River, and one of the main reasons for the population differentiation might be the construction of these dams (Heidari et al. 2013). A detailed study involving the molecular genetics and environmental aspects may further confirm the present findings unambiguously. This study provides basic information about the morphometric differentiation of *C. gracilis* populations in the upstream and downstream of Manjil and Tarik dams and suggests that morphological variations observed in *C. gracilis* should be considered in stock enhancement programs.

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